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14. ABSTRACT
Since this application was written, it has become increasingly appreciated that NFκB activation can either promote cancer cell death or cancer cell survival – the outcome being dependent on the context of parallel biological processes. Notably, the presence of tumor suppressors influences the outcome. Our bioinformatic approach to define a cancer promoting NFκB gene activation signature is proving to be well suited for accounting for the varied and opposing roles of NFκB activation. Specifically, our prostate cancer specific work has identified absence of a unique set of tumor suppressors which leads to cancer cell survival and ultimately lethal prostate cancer. Notably, PTEN was not one of these tumor suppressors. It is also now appreciated that indiscriminate inhibition of NFκB activation may be problematic as this may block the anti-cancer effect of NFκB activation. As such the increased understanding of NFκB activation's "context dependency" adds further support for the work we are doing. In year one and two of the project we have found elevated cytokines and presence of T. Vaginalis at time of diagnosis of prostate cancer are not associated with higher grade disease nor risk of relapse after prostatectomy. We have also identified a 31 tumor gene signature which correlates with relapse with lethal disease post prostatectomy in our training set. We have also used the 31 gene signature and publically available data-bases to computationally create a refined network of cancer promoting NFκB gene activation. This network is now being used to i) further inform selection of genes to test in multiple independent data sets for association with lethal disease; ii) inform and increase power for identification of new SNPs in GWAS datasets associated with lethal outcome, and iii) help to interpret the mechanisms of action of genes associated with lethal disease identified separately in the tumor gene expression profiling and SNP analyses. This work will distinguish itself from the Polaris and Genomic Health gene signatures by being able to select patients for pharmacologic intervention with NFκB inhibitors under development and hopefully obviate the need for radiation of prostatectomy.

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INTRODUCTION:

The 4% per year decline in the age specific prostate cancer mortality in the USA has come at the cost of treating a significant number of men who did not require therapy and are living with adverse events that diminish their quality of life. To identify patients with lethal prostate cancer we are deploying a "systems biology approach" to develop a risk scoring system. The systems biology approach is making use of the epidemiological, clinical, pathological and biological data that has implicated Nuclear factor kappa B (NFκB) activation in the development of lethal prostate cancer. Specifically, we hypothesize that lethal prostate cancer results from exogenous insults causing NFκB activation which sets up a vicious cycle with further inflammatory insults and culminates in sustained NFκB activation and carcinogenic changes in the microenvironment. This persistent activation results in progression of prostate cancer to a lethal disease. We aim to identify patients with lethal prostate cancer using a systems biology approach focused on the NFκB pathway which will enable the construction of a risk scoring system to identify patients with localized but potentially lethal prostate cancer in need of therapy and patient who can safely avoid therapy.

BODY:

New data since the original submission of this grant adds further support to the role for inflammation and more specifically nuclear factor kappa B (NFκB) activation in the development of cancer in general and prostate cancer specifically. More importantly it has been realized that NFκB activation can either promote cancer cell death or cancer cell survival – the outcome being dependent on the context of parallel biological processes. Specifically, our data to data has found that absence of a unique set of tumor suppressors in the context of other NFκB genes is associated with lethal outcome[1]. Notably, absence of PTEN was not one of the tumor suppressors. Moreover, drugging NFκB activation by blocking IκK (upstream of NFκB) is problematic due to non-NFκB effects[1]. It is also now appreciated that indiscriminate inhibition of NFκB activation may be problematic as this may block the anti-cancer effect of NFκB activation. As such the increased understanding of NFκB activation's "context dependency" adds further support for the work we are doing. In year one and two of the project we have found elevated cytokines and presence of T. Vaginalis at time of diagnosis of prostate cancer are not associated with higher grade disease nor risk of relapse after prostatectomy. We have also identified a 31 tumor gene signature which correlates with relapse with lethal disease post prostatectomy in our training set. We have also used the 31 gene signature and publically available data-bases to computationally create a refined network of cancer promoting NFκB gene activation. This network is now being used to i) further inform selection of genes to test in multiple independent data sets for association with lethal disease; ii) inform and increase power for identification of new SNPs in GWAS datasets associated with lethal outcome, and iii) help to interpret the mechanisms of action of genes associated with lethal disease identified separately in the tumor gene expression profiling and SNP analyses. This work will distinguish itself from the Polaris and Genomic Health gene signatures by being able to select patients for pharmacologic intervention with NFκB inhibitors under development and hopefully obviate the need for radiation of prostatectomy.

Task 1. **Identify individual features of NFκB activation which are associated with lethal disease.**

(Months 1 to 18)

Task 1A: *Perform gene profiling of tumors and determine whether a set of genes and/or proteins indicative of NFκB activation are associated with lethal prostate cancer. Data will be available at time of commencing the project on 350 patients and we will generate new data on 154 more patients. Data mining and analysis of existing data will be performed to define the 40 gene panel to be assessed for correlation with lethal disease. (Month 1 to 18)*

Accomplishments: In the first 24 months of the grant we have (i) developed putative gene sets from the initial data-mining efforts of publically available data-sets which are serving as our "discovery gene sets"; (ii) successfully deployed the "Nugen-Affy" assay as a reliable approach for whole genome expression analysis of lethal versus non-lethal prostate cancer – the "discovery gene-sets" are now being inputted into this data-set to define the "training set" and (iii) commenced creation of the Tissue Micro-Arrays (TMA) and extraction of nucleic acids for creation of an independent "validation set" of lethal versus non-lethal prostate cancer.

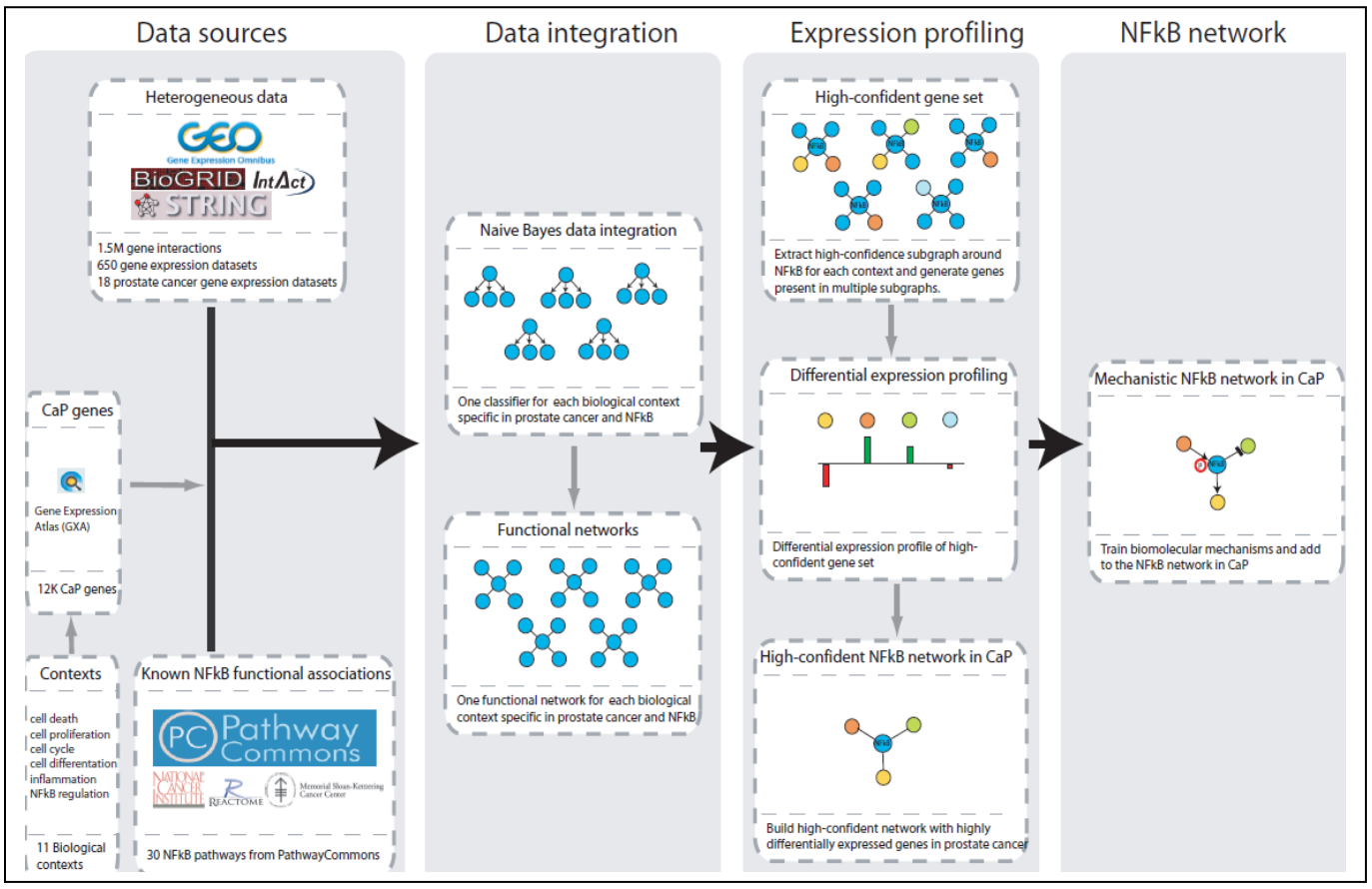


Figure 1: Schematic Summary of Data-mining Process:

- The left columns depict the mining of the 878 publically available databases which lead to the creation of the biological context specific networks.
- High-confidence subgraphs around the NFκB gene were identified in each context specific network and assessed by an additional set of hierarchical mechanism-specific learners to create a complete inferred biomolecular pathway. This identified both characterized and novel NFκB interactors in prostate cancer.
- The 351 selected NFκB related genes created the High-confidence NFκB network and 271 of these were in the 6096 gene DASL gene expression database annotated with lethal versus not lethal outcome from Physicians Health Study cohort of patients (middle figure of third panel). This was used to refine the gene set to those associated with lethal prostate cancer (31 genes).
- The 31 genes were then used to refine the network to define a network of NFκB cancer promoting genes in prostate cancer (fourth panel). See supporting data (page 23) for the genes and their corresponding context and types of interactions.

(i) Data-Mining: The research team has completed analyses of total of 878 expression and interaction datasets using context-specific Bayesian learning (Park el al 2010). These datasets included integration of 18 curated prostate cancer expression datasets and GEO[2] and ArrayExpress[3] and cancer-specific arrays from these repositories, and non-condition-specific genomic data such as physical and genetic interactions from BioGRID[4] and IntAct [5], transcriptional regulatory relationships from Transfac[6] and cisRED[7], and miRNA data from miRBase[8]. High-confidence subgraphs around the NFκB gene were identified in each and assessed by an additional set of hierarchical mechanism-specific learners to create a complete inferred biomolecular pathway. These resources provided literally billions of datapoints which have been integrated with our prostate cancer-specific clinical and genomic data, using methodologies developed by members of our research team[9-12]. Such data is key both for enriching detailed mechanistic models of prostate cancer development at the molecular level and, as has been done previously for genetic data[13,14] differentiating common functional variation in the general population from causal variation specific to lethal prostate cancer.

Using this approach 351 genes were identified for further exploration: Specifically, we focused on transcripts (i) correlated with NFκB activation in external expression data from GEO and ArrayExpress. Similar systems approaches have been highly successful in illuminating the entirety of the biomolecular pathways contributing to basic biology phenotypes in model organisms [15,16]. We contend that this integrative modeling is critical to understanding and detecting the development of lethal prostate cancer and will define a critical set of genes ("gene-panel") indicative of NFκB activation and in turn lethal prostate cancer.

The second step in this process was to define NFκB networks that correspond to distinct biological processes. We chose to develop 11 networks from a total of 442 different biological or biochemical processes. The key determinant was whether the biological context was related to cancer biology and associated with the hallmarks of cancer and in turn lethal prostate cancer or NFκB related biology. The 11 contexts analyzed were (i) cell death; (ii) cell migration; (iii) cytokine metabolic process; (iv) mesencyhmal cell differentiation; (v) positive regulation of NFκB, (vi) regulation of cell cycle (vii) regulation of cell differentiation; (viii) regulation of cell motion; (ix) regulation of cell proliferation; (x) stem cell maintenance; (xi) vasculature development. Four of the 11 networks are depicted below

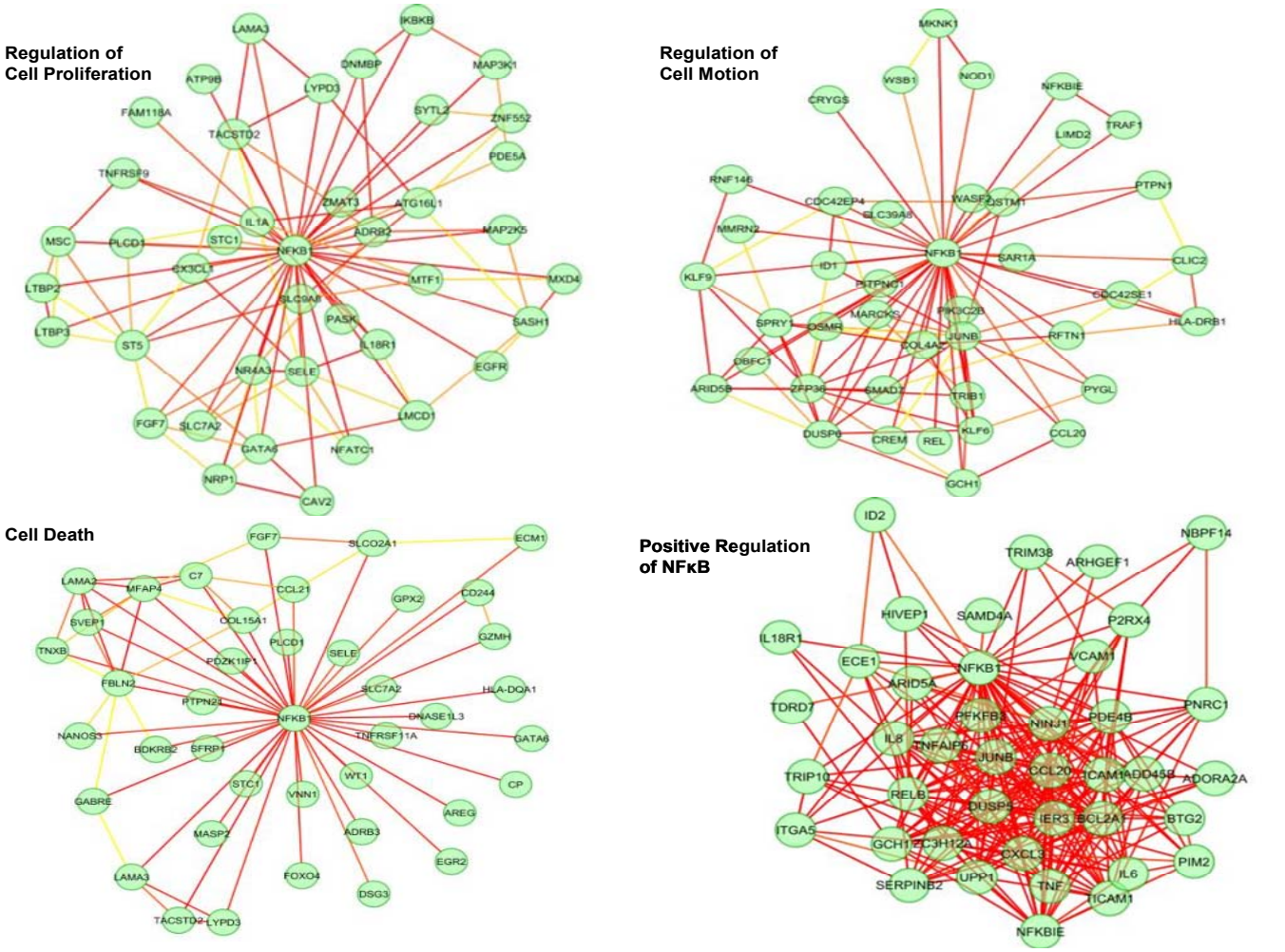
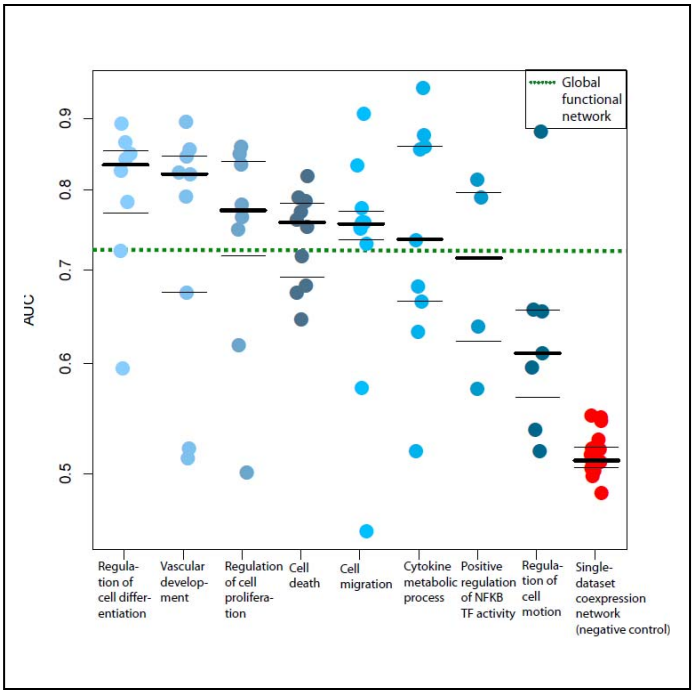


Figure 2: Representative NFκB networks

The notable findings from this preliminary work are that there are distinct networks for the unique biological processes. The team then computed for each gene the frequency of co-occurrence among all genes from these 11 networks. It was noted that NFκB1 co-occurs in all 11 context-specific subnetworks (as expected), NFκBIE in 5, CCL20 in 4. Besides NFκB1 there is no other gene that co-occurs in all 11 context-specific sub-networks.



The ability of the NFκB context specific networks to predict biomolecular mechanisms in pathway-specific functional relationship networks in prostate cancer was assessed using receiver operator curves. Figure 3 shows the performance of 8 context-specific inferred networks compared with the performance of a non-context-specific global inferred network (green dotted horizontal line) and a control of coexpression networks from 18 curated prostate cancer specific single expression datasets. 7 of 8 predictions all achieved AUCs over 0.7 for all specific mechanistic interaction types and over 0.75 for general functional associations, both using 10-fold gene-holdout-based cross-validations. The cell death network has so far included several of the highest-confidence links between NFκB1 and characterized examples such as CCL2[17] (regulatory). HDAC1[18] (phosphorylation) and IKBKB[19] (physical). This computational method easily scales to integrate thousands of experimental results and to identify those data most informative regarding specific putative mechanisms of interaction in pathways surrounding genes of interest in cancer.

Identifying NFκB related genes associated with lethal prostate cancer.
Having defined NFκB related genes in prostate cancer, we then sort to determine which genes are associated with lethal prostate cancer in a data-base of patients from the Physicians Health Study with clinical annotation connected to gene expression profile data developed from the 6,096 gene DASL platform. 217 of the 351 genes from the context specific networks were on the 6,096 gene DASL.

An assessment of the differential gene expression was done of the 217 NFκB related genes to identify genes associated with lethal prostate cancer. There were 115 PHS patients (83 indolent, 32 lethal). The DASL data is expressed as difference between the mean log expression in the lethal group and in the indolent group. When the expression is larger in the indolent group, the result has minus indicating the gene is decreased in the lethal group. Differential gene expression was inferred using package *limma*. An analysis was performed with and without Gleason grade as an additional covariate, Gleason scores are recoded as "low" (<7), "med" (=7), and "high" (>7).

Analysis **without** Gleason grade as a covariate:

- PHS subset - 186 of the 6096 genes on the DASL platform were differentially expressed between men who underwent a prostatectomy and relapsed and died of prostate cancer (N=32 lethal disease) compared with those who did not relapse after a prostatectomy (N=83: cured by prostatectomy or had disease that was indolent and did not require a prostatectomy).
- FDR correction was performed using Benjamini-Hochberg method.
- 19 of these genes were associated with the NFκB network.

	logFC	t	adj P Val	rank	observed score
FOSB	-1.22	-5.53	0.00	11.00	-1.00
ZFP36	-1.07	-5.46	0.00	12.00	-1.00
ATF3	-0.96	-5.22	0.00	15.00	-1.00
EGR2	-1.11	-5.13	0.00	18.00	-1.00
JUNB	-0.81	-4.98	0.00	25.00	-1.00
NR4A3	-1.19	-4.65	0.00	35.00	-1.00
SELE	-0.83	-4.41	0.00	46.00	-1.00
FOSL2	-0.39	-4.25	0.00	52.00	-1.00
BTG2	-0.39	-4.20	0.01	58.00	-1.00
HBEGF	-0.65	-4.11	0.01	66.00	-1.00
SFRP1	-0.79	-4.01	0.01	73.00	-1.00
NEDD9	-0.48	-3.86	0.01	85.00	-1.00
CXCL2	-0.81	-3.80	0.01	96.00	-1.00
DUSP5	-0.76	-3.72	0.02	112.00	-1.00
TRIB1	-0.35	-3.64	0.02	124.00	-1.00
CX3CL1	-0.60	-3.61	0.02	127.00	-1.00
IL1B	-0.57	-3.48	0.03	143.00	-1.00
NPR3	-0.80	-3.47	0.03	145.00	-1.00
CEBPD	-0.53	-3.46	0.03	148.00	-1.00

- Swedish subset: 129 genes are differentially expressed at 0.05 FDR level between lethal and indolent subgroups. FDR correction was performed using Benjamini-Hochberg method. 3 genes found to be significant in our analysis belong to the 271 NFκB gene set.

- It is of note, the tissue analyzed from this analysis was obtained by TURP and is disease arising from the central gland with a different biology to disease from peripheral portions of the prostate gland.
- Patients in this data-set were also managed with "watchful waiting" and did not undergo a prostatectomy.

	logFC	t	adj P Val	rank	observed score
SLC39A8	-0.35	-4.20	0.01	28.00	-1.00
SLCO2A1	0.30	4.13	0.01	30.00	1.00
KLF10	0.22	3.96	0.01	41.00	1.00

Given, the lack of uniformity in tissue collection, management and biology between the PHS and Swedish subsets, we elected to focus on the PHS cohort for creation of a discovery gene set of cancer promoting NFκB gene activation. The PHS represent the more commonly ascertained tissue of prostatectomy and TRUS biopsy.

Analysis **with** Gleason Grade as a covariate: On account of trying to improve on the prognostic ability of Gleason Score, we performed analysis with Gleason Grade as a covariate in the PHS prostatectomy series. 0 out of 6096 genes represented on a DASL platform are differentially expressed at 0.05 FDR level between lethal and indolent subgroups in this analysis. (FDR correction was peformed using Benjamini-Hochberg method). When there is no multiple testing correction, there are 384 differentially expressed genes with p-values below 0.05. 31 of these genes belong to the NFKB 271 gene set.

Gleason Score	Non-lethal	Lethal
6	15	0
7	58	9
8	7	11
9	3	9
10	0	3

	logFC	t	P Value	adj P Val	rank	observed scc
ZFP36	-0.82	-3.36	0.00	0.31	20.00	-1.00
JUNB	-0.68	-3.34	0.00	0.31	22.00	-1.00
IL1B	-0.68	-3.31	0.00	0.33	23.00	-1.00
FOSB	-0.88	-3.23	0.00	0.38	24.00	-1.00
ATF3	-0.74	-3.20	0.00	0.38	26.00	-1.00
KLF6	-0.76	-3.17	0.00	0.38	31.00	-1.00
GPX2	0.54	3.11	0.00	0.44	33.00	1.00
EGR2	-0.82	-3.06	0.00	0.45	37.00	-1.00
NR4A3	-0.95	-2.96	0.00	0.48	46.00	-1.00
OAS2	0.62	2.93	0.00	0.50	49.00	1.00
CEBPD	-0.54	-2.78	0.01	0.55	70.00	-1.00
ENPP1	-0.54	-2.74	0.01	0.57	76.00	-1.00
HBEGF	-0.52	-2.64	0.01	0.61	89.00	-1.00
CDC42EP4	-0.53	-2.58	0.01	0.61	104.00	-1.00
DUSP6	-0.55	-2.57	0.01	0.61	110.00	-1.00
ITGA5	-0.69	-2.55	0.01	0.61	115.00	-1.00
BTG2	-0.30	-2.54	0.01	0.61	122.00	-1.00
SFRP1	-0.61	-2.50	0.01	0.62	134.00	-1.00
DUSP5	-0.64	-2.50	0.01	0.62	136.00	-1.00
FOSL2	-0.28	-2.48	0.01	0.62	144.00	-1.00
SELE	-0.57	-2.46	0.02	0.62	149.00	-1.00
MKNK1	0.53	2.43	0.02	0.64	156.00	1.00
CX3CL1	-0.50	-2.43	0.02	0.65	157.00	-1.00
ARHGEF7	-0.38	-2.30	0.02	0.68	209.00	-1.00
BCL6	0.62	2.29	0.02	0.68	216.00	1.00
TRIB1	-0.26	-2.22	0.03	0.70	245.00	-1.00
IER3	-0.42	-2.11	0.04	0.72	314.00	-1.00
BDKRB2	0.43	2.05	0.04	0.74	346.00	1.00
NEDD9	-0.32	-2.04	0.04	0.76	352.00	-1.00
CXCL2	-0.53	-2.00	0.05	0.77	376.00	-1.00
FOXJ1	0.56	1.98	0.05	0.79	384.00	1.00

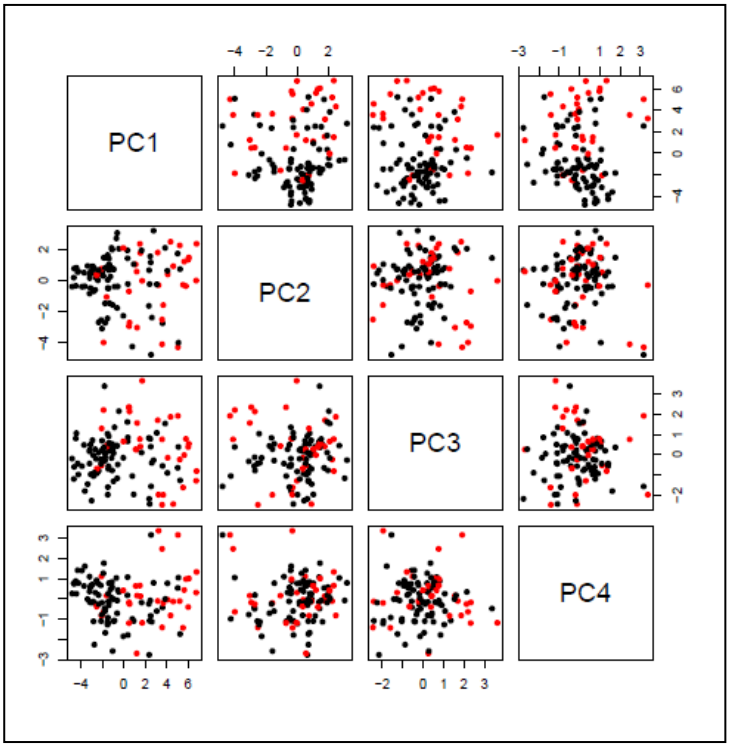
Of the 19 genes differentially expressed with FDR correction and identified without Gleason in the covariate, 18 overlap with the 31 differentially expressed genes with p value of 0.05 (but testing for multiple corrections). Notably a consistent finding is there is down regulation of tumor suppressors in lethal tumors when both types of analyses are used. The tumor suppressors identified to be lost in lethal prostate cancer are:

- *CEBPD:* CCAAT/enhancer-binding protein delta
- DUSP5: Dual specificity protein phosphatase 5
- SFRP1:Secreted frizzled-related protein 1
- NR4A3: neuron-derived orphan receptor 1 (NOR1) also known as NR4A3
- ZFP36: Tristetraprolin (TTP), also known as zinc finger protein 36 homolog

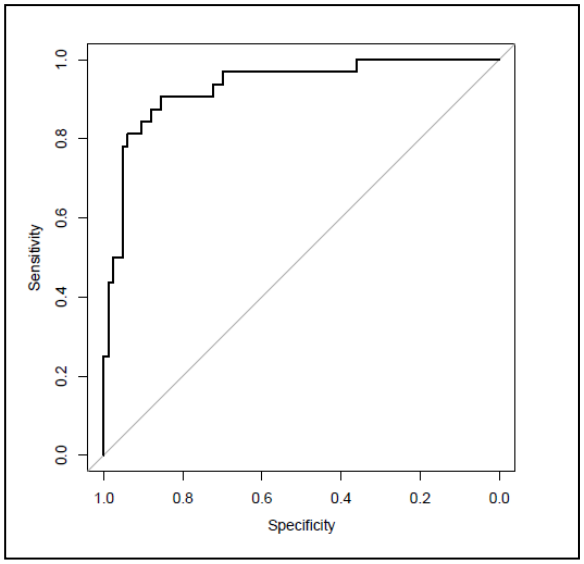
Of the 31 genes identified, 6 are increased in lethal vs non-lethal and independent of Gleason (GPX2, OAS2, MKNK1, BCL6, BDKRB2, FOXJ1). These were not found when Gleason was not in the model and FDR correction

performed. 7 genes were down regulated and independent of Gleason in lethal disease but not found with FDR when Gleason was in the model.

Principal Component Analysis PCA was performed on PHS cases using significant genes form NFκB set. PCA 1, 2 3 and 4 were developed. Logistic regression of the 4 different PCA found PCA1 and PCA3 were significantly associated with lethal cancer.



Logistics Regression Deviance Residuals				
Min	1Q	Median	3Q	Max
-2.30	-0.41	-0.20	0.31	2.77
Coefficients				
	Estimate	Std Error	z value	Pr (>|z|)
Intercept	0.47	0.48	0.97	0.32
PC1	0.41	0.12	3.55	0.0004
PC2	-0.26	0.17	-1.51	0.13
PC3	0.64	0.25	2.56	0.01
Gl Low	-18.77	1511	-0.01	0.99
Gl Med	-2.92	0.71	-.41	4.22e-05



ROC were generated and AUC of 0.9313 was found for predicting lethal vs indolent. We note that this is subject to over-fitting because the data used to generate PCA were not subject to correction for multiple testing AND the data inputted was selected to be differentially expressed. The current work is also assessing these "discovery sets" in publically available data sets for association with PSA relapse, Gleason score and lethality after prostatectomy. Preliminary findings have noted many of the genes are differentially expressed in these independent data-sets. Once we have a reliable gene test, we will use the refined "discovery set" as a "training set" using data from the 26,000 "Nugen-Affy" platform (see below). We note that the use of the publically available data-sets with recurrent PSA post treatment is different to lethal disease as not all patients with biochemical recurrence go onto die from prostate cancer. Nonetheless, although it is not death from prostate, a significant subset of them would have died of prostate cancer. So there is overlap with lethal AND there is overlap of biology – ie both have cancers that got out of the prostate at some time. What does not overlap all the time is the rapidity of growth as a metastatic lesion and/or responsiveness to hormonal therapy. The latter 2 phenotypes may make a metastatic lesion non-lethal. If the signature goes in the same direction with PSA recurrence – it is reassuring. If not it could be because the signature does not overcome the other two biological features. Our preliminary findings indicate many do go in the same direction.

(ii) Assessment of candidate genes in Training Set:

Details of "Nugen-Affy": In the original application, the Harvard School of Public Health collaborators had planned to generate gene-expression profile data using the 24,000 DASL platform from prostatectomy specimens from the Health Professionals Follow-up Study and the Physicians Health Study (to be referred to as HPFS/PHS cohort). However, at time of commencing the assays it became apparent the quality of the assay had declined and failed our pilot study. To address this concern, the HSPH have since successfully established the "Nugen-Affy" platform. This work was not being paid for by this DOD contract and there was no change to the budget or scope of work. The change will however result in access to high quality data and ensure we adhere to the original SOW. Specifically, to conduct transcript profiling in FFPE prostate cancer tissues, whole transcriptome amplification is being paired with microarray technologies. Briefly, RNA extracted from FFPE prostate cancer samples has been amplified using the WT-Ovation FFPE System V2 (Nugen, San Carlos, CA), a whole transcriptome amplification system that allows for complete gene expression analysis from archived FFPE samples known to harbor small and degraded RNA. Using a combination 5 and random primer, reverse transcription creates a cDNA/mRNA hybrid. The mRNA is subsequently fragmented, creating binding sites for DNA polymerase. Isothermal strand-displacement, using a proprietary DNA/RNA chimeric SPIA primer, then amplifies the cDNA. To prepare the amplified DNA for microarray hybridization, the cDNA is fragmented and then labeled with a terminal deoxynucleotidyl transferase that is covalently linked to biotin. The labeled cDNA is then hybridized to a GeneChip Human Exon 1.0 ST microarray (Affymetrics, Santa Clara, CA). This array contains roughly four probes per exon and roughly 40 probes per gene, assessing the expression of roughly 28,000 unique genes. The analysis plan is as foll**Assess for assay reliability.** A pilot study using 11-21 year old prostate tumor specimens and found excellent ($r > 0.95$) concordance of technical replicates and no influence of block age on expression profiles.

- **Assess "discovery set" in Nugen-Affy genomic data to define "training set".** Using the genes associated with lethal prostate from the data-mining efforts described above (discovery set) we will then assess them using genomic "Nugen-Affy" data using SAM and limma. If necessary, additional QC filtering will be applied and the genes re-queried until convergence on a consistent set of markers reproducibly predictive of lethal prostate cancer. We hope that standard sparse regression (lasso) or feature select will narrow this without loss of predictive accuracy to ~20 genes for application directly to the validation set (Gelb Center samples described below).
- **Our "training set" will be tested in data obtained from 420 samples** which is comprised of 140 patients with lethal disease post prostatectomy and 280 will be long term survivors/non-lethal outcome.
- **The gene-set chosen from the "Nugen-Affy" work will then be subjected to a multivariate analysis** and we will determine whether this gene-set/biological variable has a strong enough association with the lethal prostate cancer to be taken to the validation set. We will estimate Cox proportional hazards model with hazard ratios (HR) and the corresponding 95% confidence intervals (CIs) using, both without (unadjusted) or with (adjusted) the adjustment of factors known to be associated with lethal prostate cancer. The variables will include standard criteria – PSA level, Gleason score and pathological stage.

(iii) Validation Sets: In parallel with the above work we have been creating a unique cohort to validate whether the outcomes associated with the training set can be reproduced in an independent data-set.

Status of TMA Creation and Extraction of Nucleic Acids from Independent Cohort: Currently we have obtained the following samples from DFCI and ECOG repositories: Blocks from 90 unique patients with metastases post prostatectomy (lethal) and 110 patients without metastases post-prostatectomy (non-lethal/long-term survivors). When we perform the analysis we will use definitions that are harmonized with the HPFS/PHS cohort. Currently, 3 of the 4 TMAs have been created with each TMA laid out with 3 cores of tumor and 2 cores of benign/normal for each case. The TMAs are being made in duplicate and the cores of tumor and of non-cancer tissue are being obtained for nucleic acid studies. This will be completed June 30, 2013. Having defined the training set, we will have the genes for to guide the custom ordered Nanostring assay. The nanostring will be available July 2013.

We will apply the signature score of cancer promoting NFκB activation directly to the validation set of 77 lethal:77 non-lethal samples. We have previously calculated a C-statistic for Gleason score of 0.86. Compared to a model with Gleason alone, we estimate that we will have 80% power and type 1 error of 0.05 to detect an improvement in the ROC curve of 6% for the signature of activation (i.e increase to 92%) with a rank

correlation between models for both lethal and indolent of 0.8. A multivariate analysis will be used in determining whether a biological variable has a strong enough association with the lethal prostate cancer. We will estimate Cox proportional hazards model with hazard ratios (HR) and the corresponding 95% confidence intervals (CIs) using, both without (unadjusted) or with (adjusted) the adjustment of factors known to be associated with lethal prostate cancer. The variables will include standard criteria – PSA level, Gleason score and pathological stage. The gene-set that meets this criteria will be chosen for the risk assessment tool to be assessed in the biopsy cohorts in Year 3 (Aim 3).

It is of note that we will also be choosing the 4 most predictive genes which have robust antibodies available for immunohistochemistry studies. We will also make use of Nanostring's nCounter platform which has the potential to be developed as a routine clinical assay.

Task 1B: Perform protein profiling of circulating blood proteins and determine whether a protein or set of proteins indicative of NFκB activation are associated with lethal prostate cancer. Circulating proteins will be assessed in two cohorts of 312 patients. Samples have already been assembled and tied to clinical outcomes. (Month 1 to 18).

We have performed the following assays GROα (CXCL-1), IL-1α, IL-1β, IL-2, IL-6, IL-8, MCP-1 (CCL-2) and TNFα.

	GROα pg/ml	IL-1α pg/ml	IL-1β pg/ml	IL-2 pg/ml	IL-6 pg/ml	IL-8 pg/ml	MCP-1 pg/ml	TNF-α pg/ml
Mean	111.16	0.90	1.06	19.99	8.99	4.79	261.27	5.01
Median	83.90	0.09	0.69	14.06	5.19	3.85	243.72	4.02
Max	994.29	15.30	18.28	270.59	320.41	31.01	1957.95	113.27
Min	0.00	0.00	0.00	0.00	0.00	0.11	1.99	0.00

Study population:

Using Gelb Center Samples we assessed whether elevated levels of serologic cytokines were associated with risk of post-treatment relapse in patients treated with radical prostatectomy or radiation therapy. This allowed us to determine the prognostic value of pre-diagnostic cytokine levels for predicting post-treatment relapse (PTR). We utilized 190 patients from the Gelb Center cohort who received either radical prostatectomy or radiation therapy between 1997 and 2012 and who could be classified according to development of PTR. PTR (N=93) was defined as development of metastases or biochemical recurrence. Biochemical recurrence after radiation therapy was defined as occurrence of nadir+2ng/mL or initiation of salvage therapy if PSA failure had not occurred. Biochemical recurrence after prostatectomy was defined as the observation of two consecutive PSA values ≥0.2 ng/mL, at least 60 days after RP; or if the treatment was given when the PSA was less than 0.2 ng/mL but greater than 0.1 ng/mL. Metastatic disease was defined as metastases to lymph nodes, bone, or distance organs. Patients classified as being without PTR (N=96) were required to have at least 3 years of follow up.

Using EDRN samples we assessed whether serologic cytokines could distinguish between men with an elevated PSA and biopsy revealing clinically significant prostate cancer or indolent cancer or no cancer. 'Clinically Significant' cases are those with a biopsy Gleason score of 3+4 or higher. The desire was for 153 'clinically significant' cases; however, due to limited plasma only 86 'clinically significant' cases were obtained. The criteria for 'Indolent' cases are: GS 6, PSA < 10, no perineural invasion, no more than 50% involvement in a core, PSA density < 0.15, and 3 or less cores. Again, the goal was 153 indolent cases though, with limited plasma, 49 were obtained. The EDRN team provided 79 control cases (with no suspicion for atypia) to make up for the limited clinically significant and indolent cases.

Evaluation of serum markers:

Using pre-diagnostic blood samples, cytokines IL-1α, IL-1α, IL-2, IL-6, IL-8, GROα, MCP-1, and TNFα were assayed on the Meso Scale Discovery (MSD, Gaithersburg, MD) electrochemiluminescence (ECL) platform and MSD Sector Imager 2400 in the laboratory of Raina Fichorova at Brigham and Women's Hospital. The MSD ESL assays have been validated by comparison with traditional ELISA in a multicenter study led by Dr. Fichorova. For the ECL assay 96-well plates were designed specifically for use in the Sector Imager 2400 and supplied by MSD. The plates were first coated with a MSD blocking solution, then washed with a PBS-based buffer prepared in Fichorova Lab, and subsequently incubated with test samples and calibrators (25 µl/well) for 2 hours with shaking. At the end of this incubation period, the plates were washed, a detection antibody added for 1-2 h with shaking, and then washed again before adding MSD Read Buffer (150 µL/well) followed by reading in the MSD Sector 2400 Imager.

Statistical analysis:

Gelb Center: Patient clinical characteristics at diagnosis were summarized as numbers and percentages for categorical variables and as median and interquartile ranges for continuous variables. Serum marker levels were summarized as median and interquartile ranges according to development of lethal prostate cancer. The associations of serum markers with PTR were assessed using Fisher's exact tests using quartiles of each serum marker. Cochran-Armitage tests were used to summarize the trend of serum marker levels and PTR. We also conducted multivariable analyses controlling for age and D'Amico risk score.

EDRN cohort: The association of serum cytokines with clinically significant participant status (as compared to indolent) was graphically explored for each cytokine by boxplots. Cytokines were not log-transformed because each prostate cancer status group had ample sample size (in the least 49 indolent participants). Formally, the cytokine and clinical significance association was tested using logistic regression. The odds of being clinically significant were predicted separately by each cytokine. Clinically significant participants were compared to indolent participants to see if they had generally 'higher' cytokine expressions. The aim was to determine if there exists a protein profile amongst clinically significant participants—where clinically significant participants have more 'high' cytokines than indolent participants. For each cytokine, the threshold for being 'high' was determined as being above the third quartile of the combined indolent and clinically significant expressions. Ultimately each participant had a count of number of 'high' cytokines, and the distributions of each group were compared using the Chi-Square Test of Independence.

Results: Gelb Center: Selected clinical characteristics are presented in Tables 1 and 2 for Gelb Center and EDRN respectively

In the Gelb Center. The mean age of patients in both groups was 62 years. As expected, men in the PTR group had a higher mean PSA level at diagnosis (7.3 vs. 5.6) and were more likely to have Gleason 8-10 tumors (45% vs. 5%), at least 50% of biopsy cores positive for tumor, and high D'Amico risk score categorization (47% vs. 11%). There were few men with T3/T4 tumors in both groups (3% in PTR and 1% in no PTR group). The associations between each cytokine with respect to lethal prostate cancer are presented in Table 2. In univariate analyses, IL-2 was associated with PTR (p=0.03), but no clear trend in levels was apparent (p-trend=0.49). None of the cytokines were statistically significantly associated with outcome. In multivariable analyses, no statistically significant trends were apparent (data not shown).

In the EDRN cohort: The boxplots convincingly show no difference between indolent and clinically significant prostate cancer for any of the cytokines. The marginal logistic regressions failed to find evidence of cytokine differences between the groups (each p > 0.05). Further stratification of the clinically significant group into Gleason score 3+4 and >/= 4+3 did not help separate the clinically significant group(s) from the indolent group. The median IL 6 appears somewhat higher among GS 3+4 than among GS >= 4+3; however, there is not the expected gradient of IL 6 moving from indolent to GS 3+4 to GS >= 4+3. Also an ANOVA test for a difference in mean IL 6 among these three groups was non-significant (p = 0.075). The best fitting model from backward selection was fit with GRO-a and TNF-a, though neither p-value is less than 0.10.

Conclusions:

With only 93 cases with documented PTR, our power is limited to detect modest associations between levels of cytokines with respect to disease outcomes in the Gelb Center cohort. Although IL-2 levels were found to be statistically significantly associated with PTR, there was no apparent trend for levels being higher or lower in patients with PTR, thus providing limited prognostic utility. While we cannot rule out that pre-diagnostic cytokine levels may be important biologically for prostate cancer development and progression as suggested by previous studies, our investigation does indicate that these markers are inadequate for determining which prostate cancer patients will develop relapse after primary treatment. The EDRN cohort shows no association between any cytokine and clinically significant prostate cancer in men undergoing a TRUS biopsy for an elevated PSA. As such, the cumulative data from the Gelb Center and EDRN cohorts do not provide any evidence that cytokine levels at the time of biopsy or prostatectomy can help identify men with indolent disease and can be spared surgery or intervention. Conversely, none of these cytokines can be used to help identify men with low risk disease who are in need of an intervention to cure them of a potentially lethal cancer. Cytokines are not going to be used as part of the risk scoring system.

Table 1. Clinical characteristics of patients in the Gelb Center cohort selected according to development of post-treatment recurrence

	N with % or median (q1, q3)	
	No Post-Treatment Relapse N=96	**Post-Treatment Relapse N=93**
Age at diagnosis	62 (56, 70)	62 (55, 67)
PSA at diagnosis	5.55 (4.40, 7.35)	7.30 (5.05, 12.00)
Gleason at diagnosis		
6 or less	46 (48)	15 (16)
7	44 (46)	36 (39)
8 or higher	5 (5)	42 (45)
Missing	1 (1)	(0)
T stage		
T1	65 (68)	59 (63)
T2	22 (23)	20 (22)
T3/T4	1 (1)	3 (3)
Tx/Unknown	8 (8)	11 (12)
N stage		
N0	30 (31)	49 (53)
N1	0 (0)	3 (3)
Nx/Unknown	66 (69)	41 (44)
M stage		
M0	40 (42)	54 (58)
M1	0 (0)	1 (1)
Mx/Unknown	56 (58)	38 (41)
% of positive cores		
<33%	58 (60)	35 (38)
33%-50%	11 (11)	16 (17)
>50%	18 (19)	35 (38)
Unknown	9 (9)	7 (8)
D'Amico risk group		
High	11 (11)	44 (47)
Intermediate	44 (46)	40 (43)
Low	40 (42)	8 (9)
Unknown	1 (1)	1 (1)

Table 2 Distribution of age and covariates which define Indolent and Clinically Significant groups in EDRN cohorts (in addition assays were performed on 79 controls with elevated PSA and no cancer on biopsy).

	N	Median (Q1, Q3)
Age	**135**	**64 (59, 69)**
	N	**%**
Biopsy Gleason	NA	NA
<= 6	49	36
7	61	45
>= 8	25	18
PSA (ng/mL)	NA	NA
< 10	116	86
>= 10	19	14
Perineural Invasion	35	26
Any cores with > 50%	55	41
PSA Density < 0.15	84	62
< 4 Cancerous cores	83	61

Control samples are not listed in the Demographic Table. Also, the core percentage positive for cancer is unknown for 1 Clinically Significant case. Four Clinically Significant and one Indolent case have unknown PSA Density.

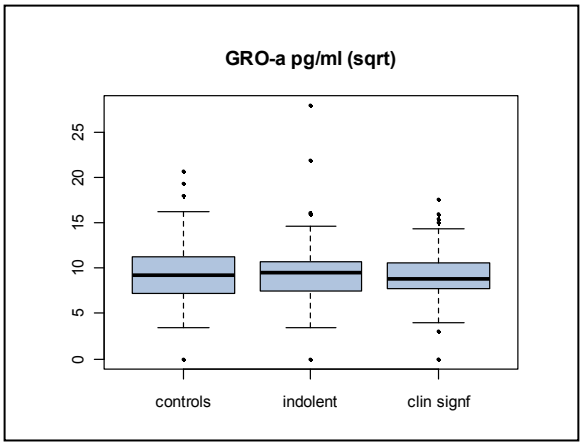
Table 3. The association between pre-diagnosticserological markers with respect to lethal prostate cancer, N=189: Gelb Center Cohort

		Post-treatment Recurrence		Univariate P-value (Fisher's Exact/Trend)
		No	Yes	
IL-1α				0.09/0.22
	Tertile 1	52 (54)	53 (57)	
	Tertile 2	15 (16)	23 (25)	
	Tertile 3	29 (30)	17 (18)	
IL-1β				0.42/0.87
	Q1	28 (29)	22 (24)	
	Q2	19 (20)	25 (27)	
	Q3	22 (23)	26 (28)	
	Q4	27 (28)	20 (22)	
IL-2				0.03/0.49
	Q1	18 (19)	9 (32)	
	Q2	31 (32)	5 (16)	
	Q3	23 (24)	9 (27)	
	Q4	24 (25)	6 (25)	
IL-6				0.34/0.67
	Q1	22 (23)	25 (27)	
	Q2	20 (21)	27 (29)	
	Q3	29 (30)	19 (20)	
	Q4	25 (26)	22(24)	
IL-8				0.13/0.35
	Q1	25 (26)	23 (25)	
	Q2	29 (30)	17 (18)	
	Q3	19 (20)	30 (32)	
	Q4	23 (24)	23 (25)	
MCP-1				0.42/0.72
	Q1	20 (21)	27 (29)	
	Q2	28 (29)	19(20)	
	Q3	25 (26)	23 (25)	
	Q4	23 (24)	24 (26)	
TNF-α				0.23/0.14
	Q1	23 (24)	25 (27)	
	Q2	22 (23)	26 (28)	
	Q3	21 (22)	25 (27)	
	Q4	30 (31)	17(18)	
GRO-α				0.42/0.49
	Q1	28 (29)	19 (20)	
	Q2	20 (21)	27 (29)	
	Q3	25 (26)	23 (25)	
	Q4	23 (24)	24 (26)	

Distribution of cytokines in EDRN cohort at time of biopsy by risk group

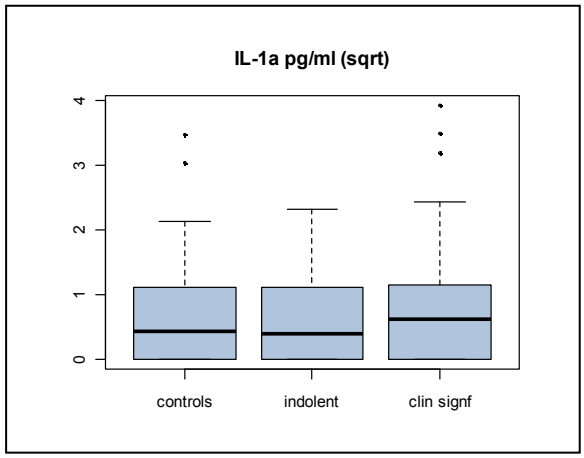
Distribution of GRO-α pg/ml

	controls	indolent	clin signf	indolent / clin signf
Min.	0	0	0	0
1st Qu.	52.52	56.79	59.9	59.24
Median	84.19	89.22	76.98	80.67
Mean	101	115.3	91.35	100
3rd Qu.	125.2	116.2	111.1	114.3
Max.	426.8	777.2	310.9	777.2



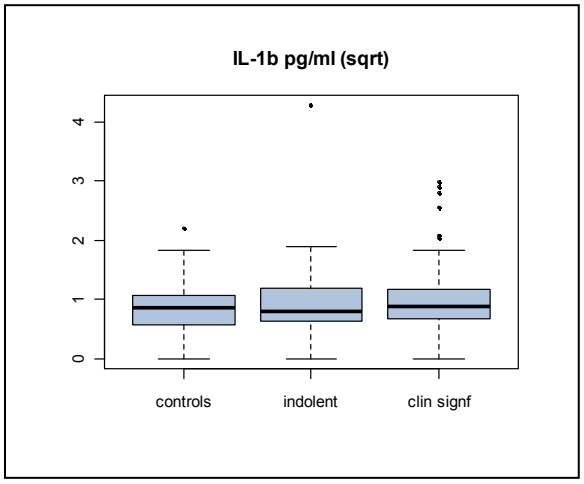
Distribution of IL-1a pg/ml

	controls	indolent	clin signf	indolent / clin signf
Min.	0	0	0	0
1st Qu.	0	0	0	0
Median	0.18	0.15	0.38	0.38
Mean	0.9885	0.9398	1.14	1.067
3rd Qu.	1.23	1.23	1.265	1.26
Max.	11.95	5.38	15.3	15.3



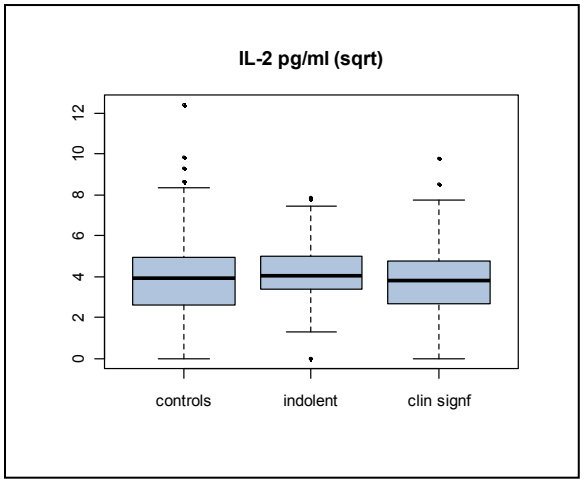
Distribution of IL-1b pg/ml

	controls	indolent	clin signf	indolent / clin signf
Min.	0	0	0	0
1st Qu.	0.33	0.39	0.4575	0.41
Median	0.75	0.62	0.775	0.77
Mean	0.9084	1.245	1.326	1.296
3rd Qu.	1.155	1.43	1.375	1.38
Max.	4.88	18.28	8.93	18.28



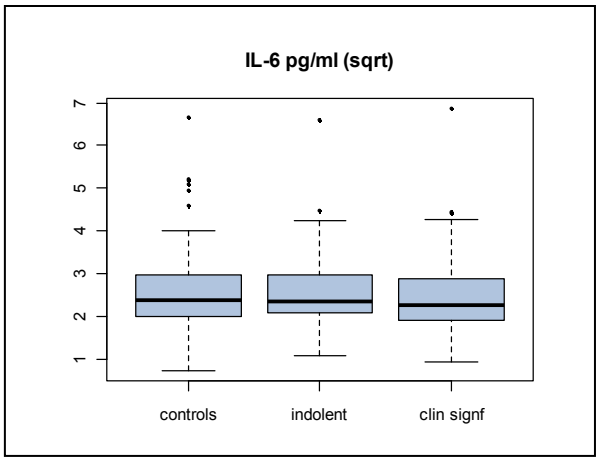
Distribution of IL-2 pg/ml

	controls	indolent	clin signf	indolent / clin signf
Min.	0	0	0	0
1st Qu.	6.82	11.32	7.22	8.935
Median	15.18	16.15	14.29	14.86
Mean	21.27	20.09	18.23	18.91
3rd Qu.	24.62	24.99	22.36	24.16
Max.	153.1	62.01	95.04	95.04



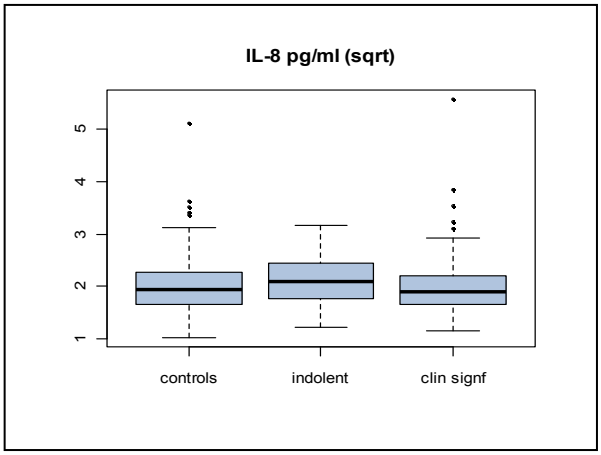
Distribution of IL-6 pg/ml

	controls	indolent	clin signf	indolent / clin signf
Min.	0.56	1.17	0.88	0.88
1st Qu.	4.05	4.43	3.66	3.94
Median	5.68	5.55	5.215	5.37
Mean	7.981	7.58	6.912	7.154
3rd Qu.	8.885	8.82	8.25	8.365
Max.	44.18	43.42	47.01	47.01



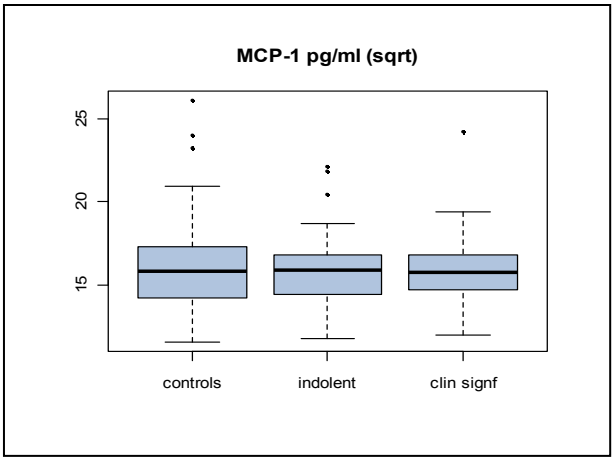
Distribution of IL-8 pg/ml

	controls	indolent	clin signf	indolent / clin signf
Min.	1.07	1.47	1.32	1.32
1st Qu.	2.785	3.1	2.752	2.875
Median	3.81	4.38	3.61	3.74
Mean	4.736	4.619	4.48	4.531
3rd Qu.	5.125	6.03	4.825	5.155
Max.	26.2	10.06	31.01	31.01



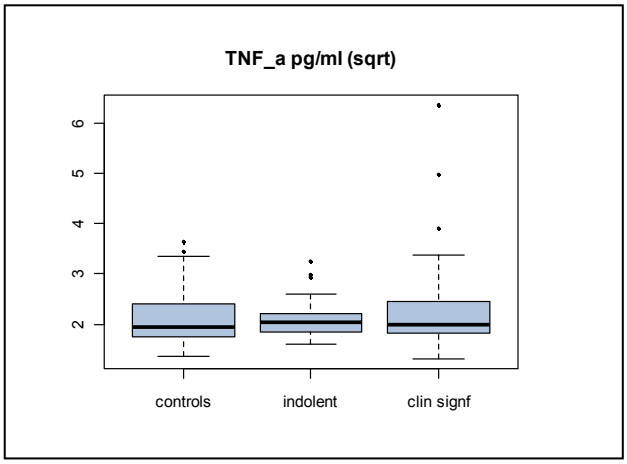
Distribution of MCP-1 pg/ml

	controls	indolent	clin signf	indolent / clin signf
Min.	134.1	138.1	143	138.1
1st Qu.	202.1	208	215.9	209.6
Median	249.2	253	247.1	250.2
Mean	264.5	255.6	252.8	253.8
3rd Qu.	299.7	283.2	282.1	282.7
Max.	680	488.7	587.2	587.2



Distribution of TNF-α pg/ml

	controls	indolent	clin signf	indolent / clin signf
Min.	1.86	2.57	1.75	1.75
1st Qu.	3.045	3.38	3.295	3.315
Median	3.76	4.21	4.02	4.05
Mean	4.744	4.487	5.49	5.126
3rd Qu.	5.825	4.91	5.932	5.195
Max.	13.32	10.59	40.34	40.34



Task 1C: *Assess whether seropositivity for Trichimonas vaginalis correlates with NFκB activation and lethal prostate cancer. We will assess the seropositivity from 111 patients with non-lethal (low risk EDRN samples) and 111 patients with lethal (metastatic, ECOG samples) prostate cancer. Samples have already been obtained and correlated with clinical outcomes. (Months 1 to 18)*

T-Vag was assayed by ELISA and provided by John Alderete of Washington State University. The assay detects IgG antibodies against recombinant T. vaginalis alpha-actinin, one of the most immunogentic trichonomad proteins. Scores of 0 (zero), 1+ and 2+ are negative. A 3+ and 4+ score are considered to be positive. Using non-DOD funds we expanded the analysis to include additional cohorts and the prevalence of seropositivity in the groups analyzed was the following

Gelb Center: N= 96 sample at radiation or surgery and no post treatment relapse: 15%

Gelb Center: N = 93 sample at time of radiation or surgery with post treatment relapse: 14%

EDRN: N=49 samples at time of biopsy and clinically indolent cancer: 12%

EDRN: N=86 samples at time of biopsy and clinically significant cancer: 15%

ECOG: N=111 samples at time of starting hormonal therapy for metastatic disease:13%

Table 1C-1. The association between pre-diagnostic serological markers with respect to lethal prostate cancer, N=189: Gelb Center Cohort

	Post-treatment Recurrence		Univariate P-value (Fisher's Exact/Trend)
	No	Yes	
***T. vaginalis* score**			0.84/0.77
0-2	82 (85%)	78 (84%)	
3-4	14 (15%)	15 (16%)	

Gelb Center T. vag positivity is in table 1C-1 and EDRN is in Table 1C-2. In neither cohort was T. vag status associated with poor risk disease. Tvag positivity did not help distinguish the association of cytokine with clinical significance status (boxplots and logistic regressions p > 0.05 – see figures on next page – Fig 1C-1).

Table 1C-2	EDRN Cohor	
	clin signf	indolent
0	73 (85%)	43 (88%)
1	13 (15%)	6 12%)

Conclusions: While the overall prevalence of *T. vaginalis* seropostivity is comparable or slightly lower than in previous studies of prostate cancer and the same laboratory was utilized for all of the published studies, only internal comparisons can be directly interpreted, as subtle changes in sample quality or laboratory conditions can impact the overall rates from one study to the next. With only 93 cases with documented PTR, our power is limited to detect modest associations between *T. vaginalis* serostatus with respect to disease outcomes. Moreover, the failure to find an association could be due to a prevalence of infection may be higher in cases without PTR than in disease-free controls, which were used as the comparison group in previous studies. There was no apparent trend for levels T. Vag levels being higher or lower in patients with PTR, thus providing limited prognostic utility. While we cannot rule out that pre-diagnostic cytokine levels and *T. vaginalis* serostatus may be important biologically for prostate cancer development and progression as suggested by previous studies, our investigation does indicate that T Vag status is inadequate for (i) determining which prostate cancer patients will develop relapse after primary treatment or (ii) help in identifying patients with Gl 6 low risk cancer who harbor more aggressive disease and require an intervention. T. vag status will not be put into the risk scoring system.

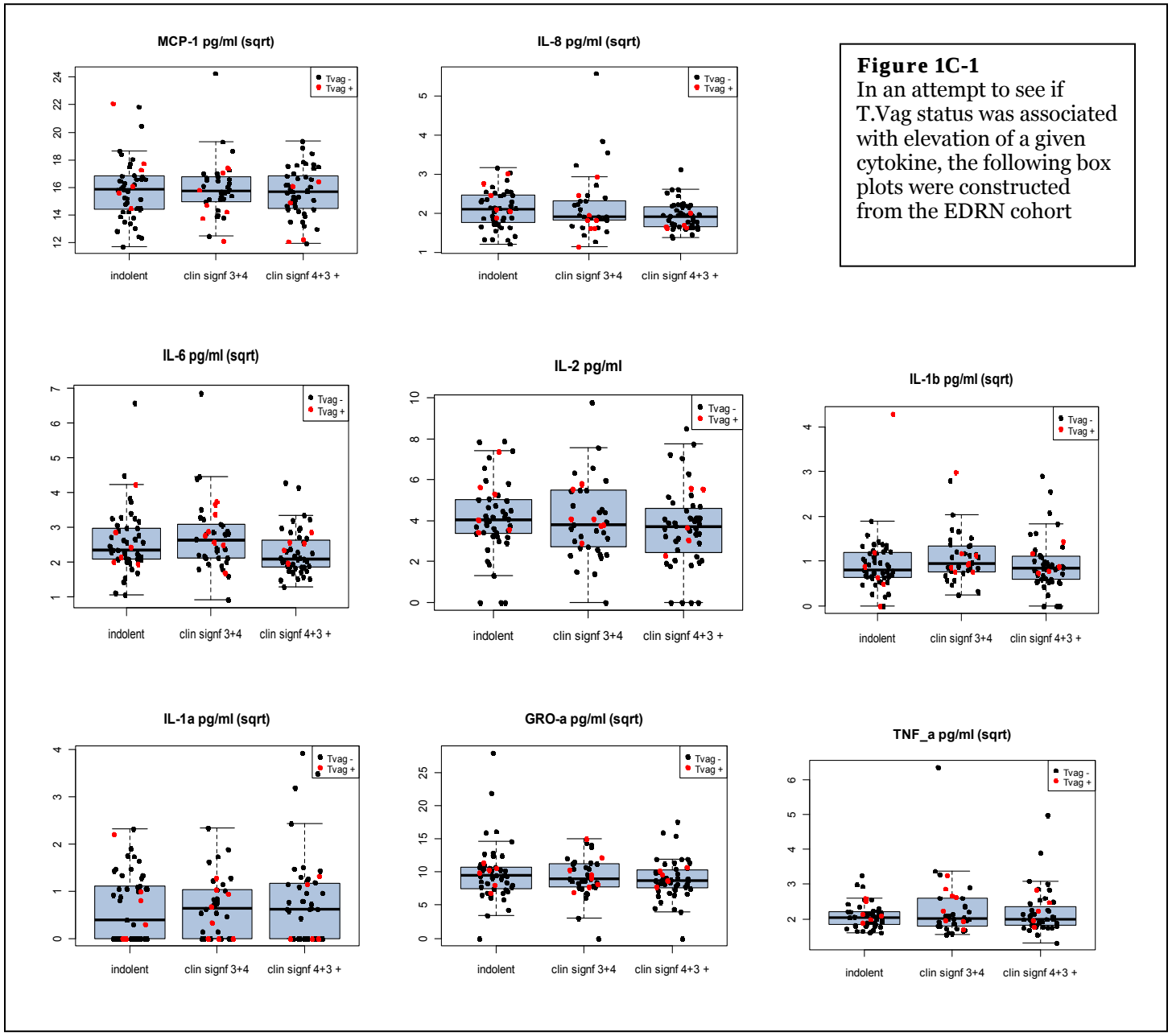


Figure 1C-1
In an attempt to see if T.Vag status was associated with elevation of a given cytokine, the following box plots were constructed from the EDRN cohort

Task 1D: *Assess whether gene variants associated with NFκB activation are associated with lethal prostate cancer. We will mine existing data sets to define the panel of gene variants to be correlated with lethal disease and then analyze 306 patients. The samples and clinical outcomes have already been assembled. (Months 1 to 18).*

Samples have been pulled and DNA isolated from the Gelb Center samples and these 153 patients will form the prostate cancer population with low risk/non-lethal prostate cancer cohort. We initially planned to use EDRN samples, but due to the samples being committed to a GWAS analysis, it was decided to use the samples in the Gelb Center. This change is being addressed with an update to the budget. The 153 samples from patients with metastatic disease (ECOG cohort) have also had their DNA extracted and are ready to be analysed. At this time, completion of this aim is contingent upon identifying the genes to be assessed. Namely, the genes will be determined from results of the work done in Tasks 1A as well as published literature on inflammation/NFκB related genes associated with prostate cancer risk[20] and prostate cancer death[21]. This will increase the pre-test probability of any SNPs associated with this work will be relevant.

We had anticipated performing the SNP analyses in by Aug 2012 (month 18 of the grant). However, due to ongoing work to create the best network (detailed in Aim1A) this part of the project was delayed. In brief, we have now created a context-specific NFκB network based on mechanistic and interaction data which

incorporated data from interrogating 6,000 DASL GEP for NFκB genes related to lethal disease. Having defined the NFκB cancer promoting activation network the team is now able to re-analyze the GWAS data. Specifically, the team is feeding the network into the established GWAS data set (Case Only Cohort: 368 indolent and 196 relapsed on Affy 5.0 SNP CHIP with 1400 CNV – which can impute ~ 500,000 SNPs). The analysis will focus on interrogating the GWAS dataset for NFκB related SNPs associated with lethal prostate cancer. Each gene in the network will receive a weight (probability) based on its functional relationship to NFκB and ranking in the network. In addition to the networks, NFκB/inflammation genes related to aggressive prostate cancer and identified in the literature[21] associated with relapse will also be feed in as prior knowledge. We will then attempt to be validated the top 40 "hits" in the case only study of 153 ECOG metastatic vs 153 Gelb Center non-lethal.

Task 2. Development of a "Risk Scoring System" for Lethal Prostate Using Multiple Factors (Months 18 to 24)

Task 2A Develop a risk scoring system from a composite of factors from Task 1 using prostatectomy cohorts to accurately identify patients with prostate cancer with a lethal potential than individual factors alone. This will be accomplished by developing a model that iteratively correlates various combinations of the different individual factors with lethal disease. We will make use of samples and clinical data from 146 patients which have already been assembled. (Months 12 – 24)

This task will be completed by month 30 of the grant – it is delayed due to being reliant on the input from Aim 1. Once the data has been established from Aim 1 (by Month 27 of the grant), a focused effort on creation of the risk scoring system over 3 months will result on completion of Task 2.

Task 3. Determine the predictive capability of "risk scoring system" using biopsy specimens. (Months 25 to 36).

Task 3A Validate the efficacy of the risk scoring system in two independent cohorts of biopsy specimens under the hypothesis that the risk scoring system will accurately identify patients with localized disease with lethal potential when using only biopsy specimens (TRUS or TURP sampling of the prostate). We will make use of samples and clinical data contained in our assembled data-bases. We will assess two cohorts of 146 patients each (total of 292 patients). Samples will be analyzed and correlated with outcome in Months 25 to 31. In months 31 to 36 the risk scoring system will be refined for maximal performance and clinical usability and manuscript written.

This task is contingent on the creation of the risk scoring system in Task 2 and collection of the biopsy specimens. We are actively collecting the Gelb Center lethal and non-lethal specimens. We have had to adopt our plan and not analyze the Swedish TURP specimens due to the inconsistency in the genomic data from TRUS and TURP specimens (secondary to different biology of centrally derived tumors and tissue processing). We have identified a cohort in the HSPH cohorts where TRUS biopsy cancer tissue and specimen for germline genomic analysis is available. We will make sure these 154 patients will be distinct from the patients analyzed as part Nugen-Affy prostatectomy series in Task 1. We will not require blood given we will not be analyzing blood borne proteins.

KEY RESEARCH ACCOMPLISHMENTS:

- Development of NFκB networks associated with different biological context and an NFκB network associated with lethal prostate cancer
- Curated publically available prostate cancer data-bases for interrogation and are currently being analyzed and will define a gene set associated with NFκB activation.
- Developed "Nugen-Affy" assay at HSPH to a point where it can be reliably analyzed.
- Created informative TMAs and nucleic acid resources for validation of work from HSPH cohorts
- Discovered that T. vaginalis status and inflammation related proteins cannot be used to distinguish patients with low grade disease who harbor a disease that is actually higher risk disease
- Set the groundwork for a robust analysis that will (or will not reliably define a gene set that identities patients with low risk disease with lethal potential – and a biology to target that may guide pharmaceutical strategies to prevent development of or progression of lethal cancer.

REPORTABLE OUTCOMES:

- manuscripts, abstracts, presentations:
  - Symposium on Systems Biology of Diversity in Cancer at MSKCC, October 18-19 2012, Memorial Sloan-Kettering Cancer Center, New York, USA."Predicting biomolecular mechanisms in complex specific functional relationship networks in prostate cancer"

  - ISMB/ECCB 2013, NetBio Satellite Meeting, July 19 2013, Berlin, Germany. "Predicting biomolecular mechanisms in complex specific functional relationship networks in prostate cancer"

- licenses applied for and/or issued: None
- degrees obtained that are supported by this award; None
- development of cell lines, tissue or serum repositories: Creation TMA and nucleic acid collection annotated with clinical outcome in collaboration with Gelb Center
- informatics such as databases and animal models, etc.: None
- funding applied for based on work supported by this award: None
- employment or research opportunities applied for and/or received based on experience/training supported by this award: None

CONCLUSION:

As detailed above, we have generated a significant amount of data and are now performing the robust statistical analyses which will lead to reliable new findings. We anticipate having findings either supporting or refuting the hypothesis that tumor and/or germline genetic profiles of inflammation/NFκB activation is associated with lethal prostate cancer. This data will be able to tell us whether germline SNP and/or tumor gene expression profiling focused on inflammation can be used as a prognostic factor in patients diagnosed with prostate cancer. Moreover, it will set the stage for applying these findings to assess whether one or more of the findings can identify patients with clinically localized disease and suitable for surveillance as well identify a biology (inflammation) or target (NFκB) to abrogate and prevent progression on surveillance and/or eradication of micrometastatic disease post definitive local therapy.

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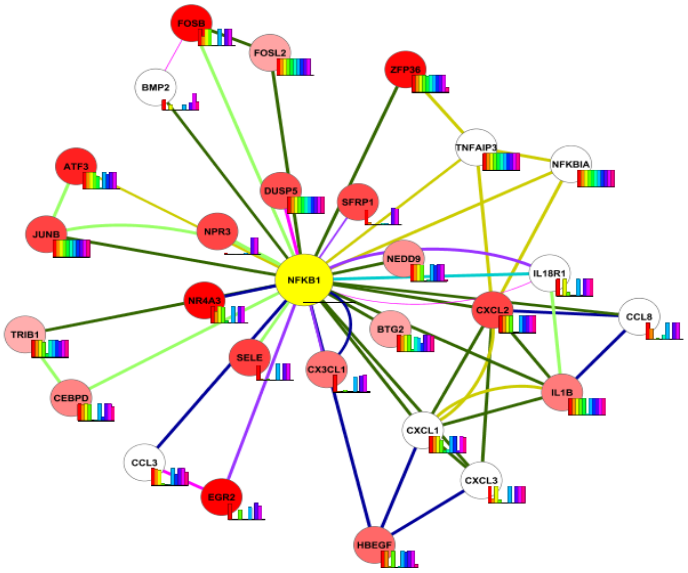
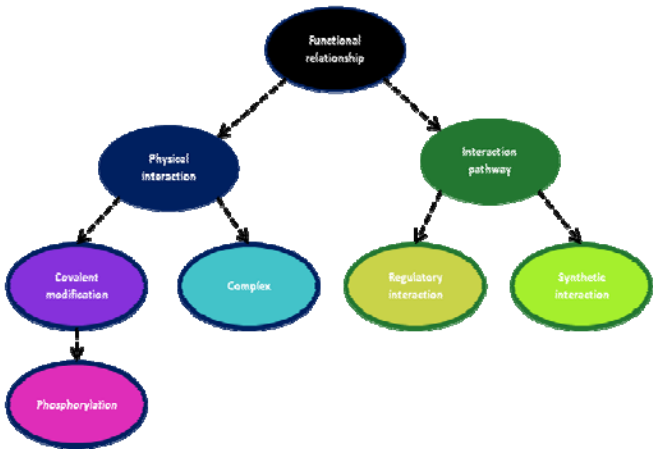
APPENDICES:

None

SUPPORTING DATA:

Gene	Predicted mechanism with NFKB1
ATF3	regulatory
BTG2	interaction pathway
CEBPD	synthetic
CX3CL1	covalent, physical
CXCL2	interaction pathway
DUSP5	phosphorylation
EGR2	covalent
FOSB	interaction pathway
FOSL2	interaction pathway
HBEGF	physical
IL1B	interaction pathway
JUNB	interaction pathway
NEDD9	interaction pathway
NPR3	synthetic
NR4A3	physical
SELE	synthetic
SFRP1	covalent
TRIB1	interaction pathway
ZFP36	interaction pathway
BMP2	interaction pathway
CCL3	physical
CCL8	interaction pathway
CXCL1	interaction pathway
CXCL3	interaction pathway
IL18R1	phosphorylation, complex
NFKBIA	regulatory
TNFAIP3	regulatory





Color scheme: contexts

- Positive regulation of Nfkb1 TF activity
- Regulation of cell differentiation
- Stem cell maintainance
- Vascular development
- Regulation of cell proliferation
- Regulation of cell motion
- Regulation of cell cycle
- Mesenchymal cell differentiation
- Cytokine metabolic process
- Cell migration
- Cell death

The top left figure details the genes associated with lethal prostate cancer in the PHS set and the colors of the lines details how the genes interact (top right panel) and the colors of the circles indicate the biological context most strongly associated with the gene